TESTICULAR RESPONSE IN BOBWHITES TO INTERRUPTED DARK PERIODS[1]

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In many photoperiod studies relating to reproduction in birds, the basic technique is the use of light to influence gonadal development and function. In Bobwhites (*Colinus virginianus*), gonadal responses to light are unequal between sexes. Incidental evidence from previous experiments (Kirkpatrick, 1955) indicated that cocks become sexually mature substantially before hens lay, when both have received identical light treatment and management. In quantitative photoperiodicity studies based upon time, intensity, or other variables, it is never implicit that results pertaining to one sex of Bobwhites apply to the other sex. Thus, experiments to test the effects of light upon reproductive response in Bobwhites, and perhaps other species, should be designed accordingly if the desired end point is a mere stimulation of tissues, full spermatogenesis, or ovulation. This paper reports an experiment to determine the time required for Bobwhite cocks to produce mature sperm under certain conditions of lighting, and compares the thresholds of sperm production and egg laying.

Methods

The procedure of the present experiment was to sacrifice cocks after regular intervals of exposure to interrupted dark periods (Kirkpatrick and Leopold, 1952) for an evaluation of testicular development stages. It was not proposed to analyze or describe the minute details of testis histology at the sacrifice intervals, but merely to correlate the intervals with the more obvious aspects of testicular development, namely, weight, tubule expansion, lumina formation, and free spermatozoa. The accepted sign of sexual maturity in the males was the shedding of free sperm into the lumina of seminiferous tubules seen in prepared testis sections. Spermatozoa in fowls require a ripening period of about 24 hours in the excurrent ducts before they are capable of fertilization (Munro, 1938); but in this study, any requirement for sperm to collect or mature in the vasa deferentia or ejaculatory ducts as an essential step in maturation was not considered.

The laying performance of hens held as cage mates of the sacrificed cocks was recorded. This permitted a comparison between the two sexes with respect to the amount of time needed for development of full sexual activity under experimental conditions.

The experiment was begun indoors in January, 1958, with 19 cock and 12 hen Bobwhites 19 weeks old which had been reared on wire and fed a standard poultry growing mash while exposed to nine hours of light per day. One week

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before experimental lighting started, 12 pairs were formed and held one pair to a cage, seven males were caged individually, and all were fed a standard poultry breeding mash. One week later, lighting was conformed with an interrupted dark-period schedule (Kirkpatrick, 1955) in which 10 hours of light were given in two increments, one of nine hours and another of one hour, the latter splitting a 14-hour dark period. Incandescent light intensity at bird level was 30–35 foot-candles and the environmental temperature averaged 60°F., with fluctuations not exceeding about eight degrees. The details of physical arrangements for cages, lighting, and daily management have been described before (Kirkpatrick, 1955).

Samples of lighted cocks were sacrificed at regular intervals. On the beginning day of the experiment, three cocks were sacrificed to establish the weight of unstimulated testes for comparison with testis weights of lighted cocks sacrificed at weekly intervals thereafter.

The number of cocks examined at each interval was chosen to make the best use of the total number available, which was somewhat less than optimum. Previous experience indicated that the longer treatments would result in more unequivocal effects, thus fewer birds were needed for the 5-week sample, since all on that interval were expected to reach full spermatogenesis. Conversely, shorter treatments, up to four weeks at most, were expected to result in unequal development ranging from threshold stimulation of the testis to full spermatogenesis. Consequently, as many birds were examined the first two weeks as during the last three weeks.

The combined weights of testis pairs were determined while fresh on a Roller-Smith torsion balance, and the larger testis of each pair was fixed in an alcohol-formalin-acetic acid mixture. Testis sections were cut and mounted with standard procedures followed by a triple stain. Microscopic inspection of sections provided information on tubule development and spermatogenesis. The average tubule diameter for each group was determined by measuring in each testis the smaller outside diameters of 10 of the most nearly circular seminiferous tubules.

Results and Discussion

A summary of previous experiments is pertinent to the present results which are given in following paragraphs. Kirkpatrick (1955, and unpub. data) found that cock Bobwhites form mature sperm before hens lay eggs, when both receive similar treatment. First eggs from unsacrificed pairs were fertile, indicating transfer of sperm before egg-laying. One pair of Bobwhites produced fertile eggs 14 days after it was placed under incandescent light of 300 foot-candles for 17 hours per 24-hour cycle. Lower intensities (0.1–100 foot-candles) induced fertile eggs on an average of 32 days of lighting in 19 pairs.

In experiments of this nature, egg fertility established the onset of cock potency as occurring roughly no later than ovulation of the particular egg. When the interrupted dark-period method was used, and intensity varied, cocks on one foot-candle had motile sperm after 37 days. Their mates failed to lay during the same period, but hens that were on 10 foot-candles did lay.

The question of how much time is required for cock Bobwhites to produce free sperm under known conditions of artificial lighting is answered by the present results within limits set by the sampling interval. A sampling of cocks at weekly intervals was intended to discover the threshold appearance of free sperm in testis tubules seen in prepared testis sections. The threshold in days for the release of sperm was 7 to 14 days for the most precocious male and 14 to 21 days for the remaining males. The essential information leading to this conclusion is given in the following testis descriptions for the weekly intervals, in Table 1, and in Figs. 1–4.

Table 1

Testis Response of Bobwhites Subjected to Interrupted Dark-period Lighting

Treatment, numbers and response	Sacrifice interval (weeks)					
	0	1	2	3	4	5
Total interruptions (hours)	0	7	14	21	28	35
Number in sample	3	4	4	3	3	2
Average testis weight						
Grams	.06	.09	.39	.80	1.30	1.16
Per cent of body weight	.04	.05	.22	.42	.72	.64
Microscopic observations						
Testes with lumina	0	0	4	3	3	2
Testes with free sperm	0	0	1	3	3	2
Ave. tubule diameter (micra)	75	84	158	188	196	194

Testis at 0 Weeks.—After a week of conditioning on nine hours daily of artificial light (at 0 weeks with respect to the stimulating light treatment), the gonads of three cocks were in the typical inactive condition (Fig. 1). Most tubules had two irregular rows of spermatogonia situated near the basement membrane. No lumina were present. Tubules were filled with a matrix in which numerous, scattered large cells with prominent nuclei were in various stages of development or degeneration. The interstitial cells packed within the intertubular spaces had circular nuclei and little cytoplasm. The large intratubular cells appeared similar to those described for passerines (Blanchard, 1941; Wolfson, 1942) and for gulls (Johnston, 1956). In more closely related young pheasants examined in late winter, primary spermatocytes were the most advanced elements, although occasional small lumina were seen (Kirk-

patrick and Andrews, 1944; Hiatt and Fisher, 1947). Since large cells have been observed in inactive testes in several species, and variously referred to as second-order spermatogonia or inactive spermatocytes (Johnston, 1956), I interpret the immature testis of the Bobwhite as conforming to the general picture seen in other birds.

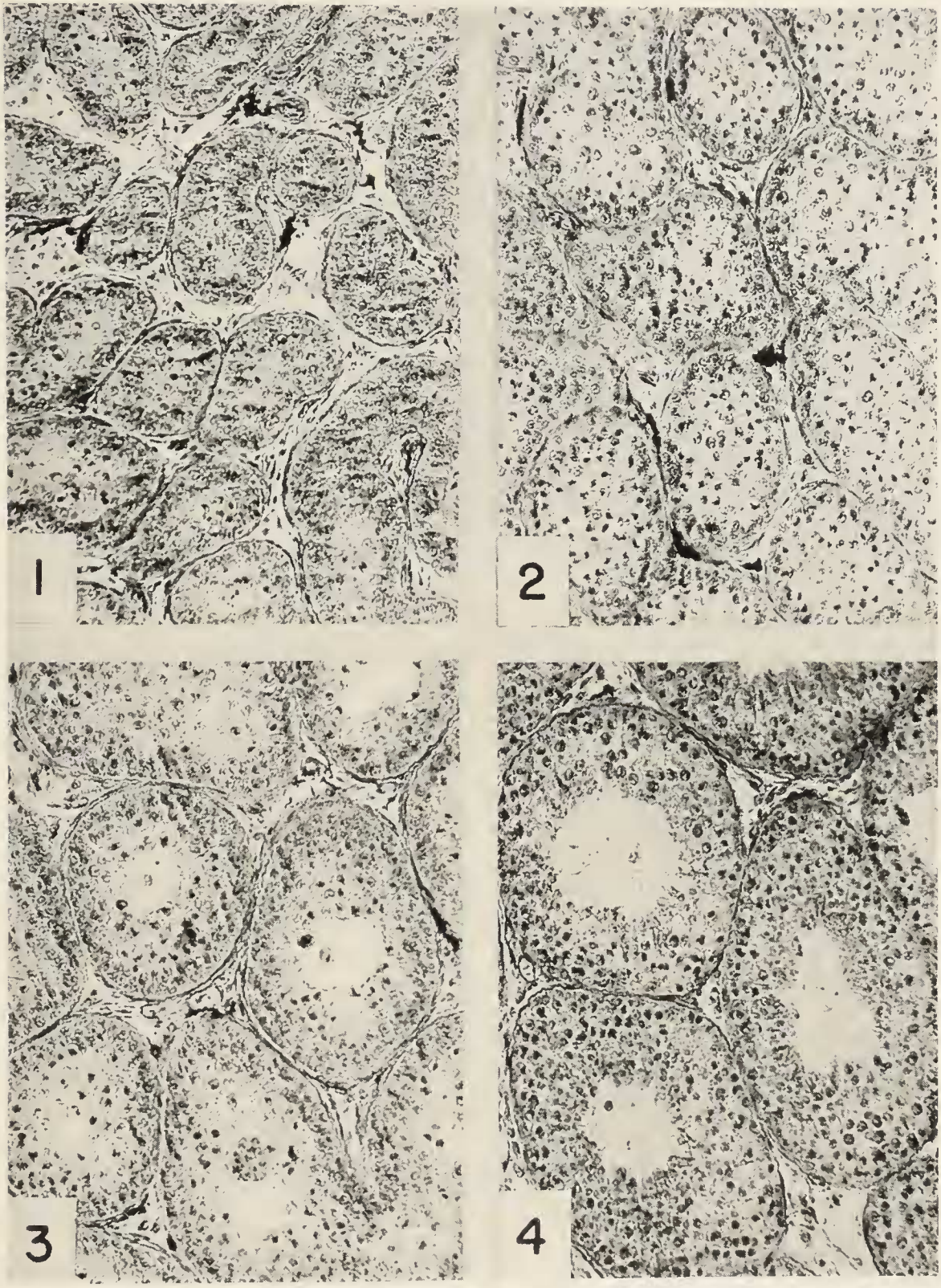
At this stage the average testis weight of 64 mgs. was lowest of the six weekly observations, and corresponded to values of 63 mgs. and 81 mgs. for unstimulated cocks held indoors under "short-day" light (Kirkpatrick, 1955). Average testis weight of unstimulated cocks of comparable age and size held outdoors was 18 mgs. in January, and 34 mgs. in March (Kirkpatrick, unpub. data). The higher values for indoor birds suggest that uniform and higher temperature may cause some increase in testis size.

Testis at 1 Week.—After seven daily 1-hour dark-period interruptions, testis weight and tubule size had increased markedly. No lumina had appeared. Some large cells were still present in the tubule matrix but primary spermatocytes predominated, many in synaptic stages. In some areas the interstitial cells were organized with quantities of cytoplasm not seen the week before (Fig. 2).

Testis at 2 Weeks.—In four individuals, new testis developments ranged from a few lumina and secondary spermatocytes to free sperm in lumina. In the most precocious bird, sperm had collected in the vasa deferentia. The average testis weight for this group exceeded a four-fold increase and average tubule diameter doubled that for the 1-week group. The absolute testis weights ranged 0.26–0.46 grams, with the more advanced spermatogenesis found in the heavier testes. Interstitial tissue was apparently reduced as a whole, but this might have been a mere flattening and redistribution among enlarging tubules as suggested by Rowan (1929) for juncos.

In one bird (Fig. 3), lumina were just opening in some tubules. In another, development had proceeded a bit farther with correspondingly more lumina and a few secondary spermatocytes which were absent in the former. In the third bird, most tubules had sperm bundles attached to the peripheries of wide lumina, but free sperm were absent. The remaining bird had free sperm in most tubules, and sperm had accumulated in the vasa deferentia.

Testis at 3 Weeks.—Testis weights doubled those of the previous sample and tubules were markedly larger. A uniform level of spermatogenic development prevailed throughout the group. All birds had free sperm in some tubules with lumina of most remaining tubules lined with sperm bundles and spermatids (Fig. 4). Some tubules in one testis were clogged with cellular debris. This phenomenon indicated arrested development of single tubules rather than regression of the whole testis, since spermatogenesis was proceeding actively in some tubules and the organ was not of maximum size.



FIGS. 1–4. Cross Sections of Bobwhite Testes, all ×175. FIG. 1. Bird sacrificed at start of experiment after lifetime treatment of nine hours light–15 hours dark. FIG. 2. Bird sacrificed after one week of interrupted dark-period treatment. FIG. 3. Bird sacrificed after two weeks of interrupted dark-period treatment. FIG. 4. Bird sacrificed after three weeks of interrupted dark-period treatment.

Testis at 4 and 5 Weeks.—At the end of four weeks all cocks were sexually functional and maximum testis weights for this experiment were reached. A slight decrease in testis measurements at the final interval is the result of natural variation, as one bird at the 5-week interval, although fully developed, had a lower tissue weight and ratio than any of the 4-week birds. Both groups may be considered as one since all birds were shedding sperm and their testis measurements, uniformly greater than those for the preceding groups, were essentially similar. In terms of function, one precocious male attained full development at two weeks with testis tissue weighing 0.46 grams, and all males were mature in the 3-week group with average testis expansion to 0.8 grams. In previous experiments, I found that Bobwhite testes of 0.3 grams or more usually contain free sperm, and that continued light stimulation caused additional growth to a maximum of 2.1 grams or 1.2 per cent of body weight. The average testis size for 14 cocks after receiving 17 hours daily light (intensities not uniform for all) for 44 days was 1.46 grams (Kirkpatrick, 1955, and unpub. data). When the average testis weight for the 4- and 5-week groups are combined, the resulting value of 1.2 grams shows that testis growth had approached the maximum size recorded for larger samples subjected to more hours of light.

Production of Sperm vs. Eggs.—The laying records of hens kept with the males used in this experiment afforded a comparison with sperm production to determine differential timing of sexual maturity. The comparison is rough because the events compared are not analogous. Free sperm as seen in testis tubules are not analogous to laid eggs, but rather to ovulated ova. Ovulation antedates oviposition by some hours. The time between ovulation and laying, unknown for quail hens, is about 25 hours in the domestic fowl (Warren and Scott, 1935). In this experiment the smallest difference in production thresholds for sperm and eggs was 26 days, more or less. The largest difference was 47 days. Hence, a delay of one or even two days by the quail ovum in the oviduct does not alter the difference significantly.

The first egg was laid 61 days after lighting started. Four hens laid their first eggs on an average of 66 days. Nine hens under observation laid their first eggs in 61 to 109 days (average 80 days). The sperm records made by dissection were in contrast to the egg records. Sperm were found in testes and in vasa deferentia at 2-, 3-, 4-, and 5-week intervals. All males examined after three weeks of lighting had sperm. Therefore, cocks shed sperm in one-half to one-quarter of the time required for hens to lay eggs.

In other experiments differing from the present one with respect to duration, age of birds, and various environmental conditions, first eggs produced were fertile (Kirkpatrick, 1955, and unpub. data). Although the cocks were not examined periodically to establish the earliest presence of sperm, the avail-

able evidence supports the conclusion that, when cock and hen Bobwhites are subjected to similar experimental conditions, the average cock precedes the average hen in sexual maturation.

Summary

When immature Bobwhites were subjected to interrupted dark-period lighting, testis weights and tubule diameters increased, but no lumina had formed after one week of lighting. Testis sections showed that one cock had free sperm in its testes and vasa deferentia after two weeks, and all cocks examined at three, four, and five weeks had responded similarly. The threshold for free sperm production was between 7 and 14 days as contrasted with the first egg at 61 days by a hen on the same treatment. When lighted Bobwhites have produced fertile eggs in much shorter periods, the experiments were not designed for determination of the time differential in sperm and egg production.

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